Research in Cell Biology 2014, 2(1): 8-16
DOI: 10.5923/j.cellbiology.20140201.02

# Bacterial Small RNAs and Their Role in Stress Tolerance and Adaptation to Environment

**Palmiro Poltronieri[1,*], Valeria Mezzolla[1,2], Oscar Fernando D'Urso[2]**

[1]CNR-ISPA, Institute of Sciences of Food Productions, National Research Council of Italy, Lecce, Italy
[2]Bioesplora srl, San Michele Salentino, Italy

**Abstract** The review recapitulate the current knowledge on the roles and importance of non-coding RNAs (ncRNAs) and small RNAs in bacteria. Many new RNAs have been described either in Gram positive and Gram negative bacteria, and in particular RNAs involved in pathogenesis and tolerance to stresses. The review recapitulate the current knowledge on the roles and importance of non-coding RNAs (ncRNAs) and small RNAs in bacteria. Several cases of regulating and signaling RNAs are presented either in Gram positive and Gram negative bacteria, with a focus on bacterial RNAs involved in pathogenesis and stress responses. Examples of small RNAs and their mechanisms of action, transcriptional and post-transcriptional regulation, induction of new genes, increased stability of their targets or their destination to the degradation pathway.

**Keywords** RNAs, Secondary structure, RNase, Binding proteins, Competition for binding, Sequestration, Adaptors

## 1. Introduction

In the November edition of Research in Cell Biology, the special issue on "RNA, signaling and RNA complexes" has launched a call for contributions on the role and importance of non-coding RNAs in eukaryotes, viruses and bacteria. Until recently, research on the regulation of bacterial physiological processes had focused mostly on the functional role of proteins. One of the aim of this review is to present regulatory RNAs and few of the new findings on their involvement in pathogenesis control, considering also other mechanisms such as response and adaptation to bacterial stress or changing environment. Current studies have reported a growing number of small RNAs (sRNAs) that play key roles in the regulation of fundamental adaptive processes like cell-cell communication (quorum sensing), transition to stationary phase, iron homeostasis and bacterial virulence.

Bacterial RNA has been shown with a role in intracellular gene regulation and communication between pathogenic bacteria and host cells in evasion of immune surveillance.

In the following paragraphs a focus on Gram negative and Gram positive bacteria non-coding RNAs will be presented, focusing on cases of involvement in pathogenesis and environmental stress, and on the mechanisms of their activity. In the last years a great progress has been made on understanding the function of bacterial RNAs. A joint ESF workshop, was hold in 2007 in Carry Le Rouet, in the frame of EU projects on RNAs, such as "Function of small RNAs across kingdoms - FOSRAK", "Non-coding RNAs in bacterial pathogenesis -BACRNA", "Novel non-coding RNAs in development and disease - RIBOREG, CALLIMIR, and RNABIO, with the participation of many RNA scientists. Thereafter, in 2009, an EMBO workshop introduced the ERA-NET Pathogenomics and sncRNAomics projects. More recently, several meetings have focused on RNA at various levels. In July 2014, in Salzburg, the meeting "DNA habitats and its RNA inhabitants" focused on viruses, transposons, introns, rybozymes and non-coding RNAs. RNA agents have been shown to play essential roles in evolution and regulation in all DNA/protein based life: based on RNA stem-loop secondary structures (built of paired stems and not-paired loops), pseudoknots, and loops with sequences showing affinity for target proteins. Infectious RNA sequence manipulate host genomes for selfish purposes and deregulation of the host cell machinery. RNAs may extend secondary structures (scaffold RNAs) that enable the interaction with several proteins to assemble protein complexes (Guttman and Rinn 2012). sRNAs often need the assistance of an RNA chaperone to correctly pair with their targets along complementary sequences (Bordeau and Felden 2014).

Bacterial small RNAs -approximately 50–250 nt in length- are generally encoded in *trans* and interact with their target mRNAs owing to imperfect or short complementarities. Therefore, one sRNA modulates the

* Corresponding author:
palmiro.poltronieri@ispa.cnr.it (Palmiro Poltronieri)
Published online at http://journal.sapub.org/cellbiology

stability and/or translation of several different target mRNAs in response to environmental changes (Geissmann et al. 2009). Their function in many cases requires the RNA chaperone Host Factor for Qβ phage RNA replication (Hfq). Hfq, an hexameric protein with two RNA recognition surfaces, one for sRNAs and the second for mRNAs, was shown to protect sRNAs from degradation and to facilitate their interaction with target mRNAs (De Lay et al. 2013). Hfq has been shown to exert a crucial role in virulence and the underlying regulation is triggered by sRNAs.

## 2. Ribonucleases

In bacteria, in addition to there are several RNases involved in RNA degradation and turnover: cas endonucleases, involved in adaptive bacterial immunity against bacteriophages; RNase III, a Mg++ dependent endoribonuclease that cleaves double stranded RNAs; two different exoribonucleases. RNase E is an exo-ribonuclease acting on single stranded RNA independently of the presence of a 5' phosphate; and polynucleotide phosphorylase (PNPase), a 3'- and 5'- exonuclease that associates to RNA degradosome in Gram negative bacteria (Guillet et al. 2013). In *S. aureus* and in *Bacillus* spp. the RNase Y is a functional homolog of RNase E, and the degradosome includes RNases J1/J2, the RNA helicase CshA, the terminal PNPase, and RNase P (Guillet et al. 2013). Bacteria contain Rsr, a ring-shaped protein that binds sRNAs such as Y RNA. Rsr and the exoribonuclease polynucleotide phosphorylase (PNPase) form an RNA degradation machine that is scaffolded by Y RNA. This sRNA, by tethering a protein cofactor, can alter the substrate specificity of the ribonuclease (Chen et al. 2013).

## 3. Toxin-antitoxin Systems

In addition to toxin-antitoxin pairs formed by proteins, modules such as protein toxin/antitoxin RNA (TA) (Fozo et al. 2008) have evolved, that assure identity and stage of growth to bacterial communities (Unterholzner et al. 2013). Gisela Storz (Bethesda, MD, USA) has described several bacterial small RNAs (Storz et al. 2011) and novel antitoxin RNAs which repress the synthesis of hydrophobic proteins toxic for the bacteria when at high levels (Fozo et al. 2010; Goeders and Van Melderen 2014). Increasing numbers of mRNA–sRNA pairs have been described, classified as type I toxin–antitoxin pairs. Type I and III antitoxins are small RNAs, often in antisense orientation, that either bind to Shine-Dalgarno region, or Transcription Initiation Sites (TIS) (Kawano 2012, Fozo 2012), or inhibits its translation (Jahn and Brantl 2013), or mediates its decay (Jahn and Brantl 2013; Wen and Fozo 2014; Wen et al. 2014). The expression of some of the toxins is induced by stress, implying that the proteins might have a beneficial role in the stressed cells. Toxin/sRNA pairs have been described in *B. subtilis* (Durand et al. 2012, Jahn and Brantl 2013) in *S. aureus* (Zorzini et al. 2014), in EHEC type *E. coli* (Wen et al. 2014); in *H. influenzae* (Ren et al. 2012) and in other pathogenic bacteria (Bertrand and Schuster 2014). In some cases the antitoxin sRNA is encoded in cis, as in the case of SymR, in cis to an SOS-induced gene that show homology to MazE (SymE) an mRNA interferase known as dormancy inducer (Kawano et al. 2007). MazE and its sRNA antitoxin have been implicated in stress induced cell death (Engelberg-Kulka et al. 2005). T.K. Toxin-antitoxin systems have been implicated in formation of biofilm and persister cells, and in the general stress response (Wang and Wood 2011).

In type II, IV and V toxin-antitoxins systems, antitoxins are proteins that are transcriptional regulators, or that are able to sequester the toxin (Syed and Levesque 2012, Wen and Fozo 2014), often are RNases themselves (Wang et al. 2013). In few cases the toxin is not an RNase but a DNase, and in this case the sRNA antitoxin originating from a cryptic prophage requires the Hfq of *E. coli* for its stabilisation (Guo et al. 2014).

## 4. Carbon Storage Regulation (Csr) System

The Carbon Storage Regulation (Csr) system is formed by CsrA, a transcriptional activator/repressor, and several sRNA components. CsrA is an RNA binding protein acting as a dimer, known in all bacteria as a global translation repression protein, having pleiotropic effects.

RNA species have been found regulating gene expression in *cis*, such as riboswitches, and in *trans*, such as enhancer RNAs; RNAs may control transcripts translation, by binding and occluding ribosome binding sites, Shine-Dalgarno sequences, Transcription Initiation Sites (TIS) and gene promoters, or switching between two genes within a bacterial operon (Gruber and Sperandio 2014).

RNAs may function as molecular mimics (Romeo et al. 2013), decoys and RNA sponges (Lapouge et al. 2013; Duss et al. 2014a), and are involved in Carbon storage regulation (Csr) / Regulation of secondary metabolism (Rsm) responses (Yakhnin et al. 2007; Marden et al. 2013; Hoe et al. 2013; Gruber and Sperandio 2014), sequestering other transcripts by means of complementary sequences; RNAs may function as adaptors, that may protect other RNAs from recruitment and cleavage by RNase III and RNase E (Duss et al. 2014a).

CsrA is also involved in repression of expression of certain genes during the stationary phase by binding to the leader sequences and ribosome binding sites of several target genes (*glgC*, *cstA*, *pgaA*). A major role of CsrA occurs during the change in gene expression during the transition from the exponential growth phase to the stationary phase. During the phase change, as a consequence of the sensing of metabolites limitation, CsrA is released from the tight binding to the Shine-Dalgarno sequence of *Hfq* gene, thus allowing its translation.

In several species, sRNAs and proteins regulating the CsrA/RsmA activity have been identified. In *Bacillus* spp., RsaE (similar to CsrA) is involved in translation initiation of the gene *hag* coding for flagellin, while the protein FliW sequesters it by binding to CsrA, thus preventing CsrA activity (Romeo et al. 2013). Members of the CsrB family of noncoding regulatory RNA molecules contain multiple CsrA binding sites and function as CsrA antagonists by sequestering this protein (Babitzke and Romeo 2007, Romeo et al. 2013).

In *Salmonella* spp., CsrA is sequestered by two sRNAs, CsrB and CsrC (Duss et al. 2014b), removing in this way CsrA repression of ribosome binding sites on target genes. In *Pseudomonas* spp., RsmE (CsrA homolog) performs translation repression on ribosome binding sites of target genes. An RNA sponge, RsmZ, is a functional homolog of CsrB and CsrC, that binds and inactivates CsrA. Another sRNA, RsmE, interacts with RsmZ thus inhibiting RNase E to perform the cleavage of RsmZ (Duss et al. 2014b).

## 5. RNA Interference and Immunity

The adaptive bacterial immunity based on RNA-guided DNA cleavage resembles the RNA interference and the PIWI associated piRNAs small-interfering RNAs (siRNAs) present in the other life kingdoms.

Drs. Emmanuelle Charpentier and Jennifer Doudna understood that, in bacteria, RNA molecules transcribed from Clustered Regularly Spaced Palindromic Repeats (CRISPR) mediate adaptive immunity against viruses and foreign plasmids. They discovered the CRISPR- encoded RNAs forming dual-RNA structures that guide the CRISPR-associated nuclease Cas9 to degrade invading DNA molecules in a sequence-specific manner. CRISPR research led up to the discovery of the mechanism of RNA-guided DNA cleavage in adaptive bacterial immunity. In Archaea, the CRISPR system is constitutive instead of inducible as in bacteria. CRISPR-Cas systems provide a mechanism for prokaryotes to incorporate short stretches of foreign DNA ('spacers') into their genomes to archive sequence information on 'non-self' DNA the bacteria encountered, such as that of viruses or plasmids. This is the adaptation stage of the immune process. Once integrated, these spacers serve as templates for the synthesis of RNA then directs Cas nucleases to specific foreign nucleic acids in order to degrade them (Westra et al. 2012). Several different types of CRISPR systems have been identified, and each is associated with a distinct set of Cas proteins (Makarova et al. 2013). Only two proteins, Cas1 and Cas2, appear to be strictly conserved among the various CRISPR systems, and they are both metal-dependent nucleases. The structure of the Cas1-Cas2 complex from *E. coli* strain MG1655 has been determined.

In addition, intriguing questions have been raised by new reports (Krupovic et al. 2014) on casposons, a superfamily of archaeal and bacterial mobile elements predicted to rely on Cas1 for integration and excision, via a mechanism similar to the integration of new spacers into CRISPR loci. Two types of nuclease are often associated with casposons, the Cas1 proteins and usually an HNH nuclease. Recurrent helix-turn-helix (HTH) domains have been identified within casposons (for example, all the Cas1 proteins of casposon family 2 have a conserved HTH appended to their C-termini), and these may play a role in the recognition of transposon ends or a target site.

In *Listeria monocytogenes* and *L. ivanovii* strains, there is a CRISPR/Cas operon, and a different CRISPR system, CRISPR-RliB (RNA in Listeria B) without any cas gene in the proximity (in *cis*), relying on polynucleotide phosphorylase (PNPase) as processing 3'–5' exonuclease or other *cas* genes in "*trans*" (Sesto et al., 2014) from a second CRISPR system. CRISPR-RliB contains 29 nt repeats spaced by 35-36 nt sequences, and has a secondary structure consisting of base pair interactions between the repeat sequence and the adjacent spacer. *L. ivanovii* contains an *RliB* gene homologue to *L. monocytogenes* and a *RliBiv2* carrying another series of repeats, with two 29nt long repeats, that is not present in *L. innocua* genome (Mandin et al. 2007, Izar et al. 2011).

## 6. Sigma Factor-Dependent Transcription

Several initiation factors (sigma factors) for bacterial RNA polymerase (RNAP) have different specificities for promoter recognition, to initiate strand opening. In *E. coli*, the housekeeping and founding member, σ70 has a recognition mechanism of the cognate elements at -10 and –35 bases that are melted by two sites that bind to A-11 and T-7 bases after DNA is unwound by transient strand opening (Darst et al. 2014). RpoS, also named σS, is a stationary phase/stress sigma factor that controls global adaptive responses to survive starvation and stress, a master regulator of the General Stress Response (GSR). The *E. coli* stress sigma factor RpoS has been shown to be a hot spot for regulation by sRNAs (Mandin and Guillier 2013). In *E. coli* the regulation of rpoS mRNA is dependent on post-transcriptional regulation involving the activity of sRNAs in cooperation with Hfq. RpoS-dependent sRNAs are known to tightly regulate the synthesis of major outer membrane proteins, such as porin OmpD. In *Salmonella* spp and *E. coli*, *OmpD* and *OmpC*, respectively, are blocked from translation during the envelope stress (Vogel 2009), by a small RNA (SdsR /RyeB) controlled by RpoS (Frolich et l. 2011), and by RybB and MycA, activated at stationary phase and by the envelope stress-activated epsilon sigma factor σE (Viegas et al. 2011, Felden et al. 2011).

## 7. Stress Tolerance: Response to pH, Temperature, Oxygen

Sigma factors such as σE, involved in envelop stress

response (causing accumulation of misfolded proteins), such as ethanol stress, hyperosmolarity and heat shock, have diverged to recognize other bases at position -10 (Darst et al. 2014). σE is present in *E. coli* but is sequestered at the plasma membrane by an anti- σE factor, RseA, that is released in response to the synthesis of the alarmone Guanosine tetraphosphate (ppGpp).

SigmaB dependent transcription occurs during oxidative stress. SbrA (Nielsen et al. 2008) and SbrE (also known as *rli47* in *Listeria*) are two sigmaB dependent sRNAs (Oliveira et al. 2009; Muhajid et al. 2013) expressed also during stationary phase of growth. Pleiotropic effects and redundancy of activity have not allowed a direct analysis of gene deregulation in the mutants in these sRNAs.

Survival of pathogens in environment and within cells is made possible by the presence of RNA sequences acting as environment sensors. Several stresses are sensed and activate a response in bacteria. At high pH, an RNA region preceding the alx gene acts as a pH-responsive element, which, in response to high pH, leads to an increase in *alx* expression in *E. coli* (Nechooshtan et al. 2009).

RNA thermometers are located within the 5'-UTR of an mRNA and form a secondary structure that occludes the Shine-Dalgarno sequence from binding to the 30S ribosome subunit (the switch off state). Temperature changes open the RNA secondary structure, thus the translation initiation complex may form or may be precluded, an interaction may occur or a signal or an effector molecule is activated. RNA thermometers are needed in the cold shock response. Several psychrotrophic bacteria may survive at cold storage condition, such as *L. monocytogenes*, *Pseudomonas fragi*, deteriorating refrigerated milk, and strains belonging to *Enterobacter, Flavobacterium, Bacillus, Clostridium, Corynebacterium, Microbacterium, Micrococcus*, and in coliforms.

For instance, cold shock protein A (cspA) is an RNA chaperone that binds to single stranded RNAs to prevent formation of secondary structures. CspA is an anti-terminator of transcription that facilitates read-through of genes induced by cold shock. The expression of cspA is upregulated during cold shock, due to the presence of two sequences located in the 5'-UTR, that may form alternate, mutually exclusive structures, able to increase the stability of cspA mRNA and its translation at 10 ℃ compared to 37 ℃ (Hoe et al. 2013).

DsrA is a small RNA expressed in *E. coli* at low temperature, that acts as an RNA thermometer and positive regulator for the stress induced RpoS regulon (Hoe et al. 2013). The translation-initiation region of rpoS forms a secondary structure that prevents efficient ribosome binding. The double-stranded region within this structure provides a target site for RNase III cleavage, leading to the degradation of the mRNA. At low temperatures, the level of the sRNA DsrA increases and it binds to the rpoS leader in cooperation with Hfq. The binding disrupts the inhibitory structure and allows ribosome access and translation of rpoS. In addition, the formed mRNA–ncRNA duplex provides a new RNase III cleavage site, that leads to the degradation of DsrA (Resch et al, 2008).

In *Salmonella* spp., the 5'-UTR of aggregation suppressing A (agsA) is regulated by temperature since it carries an RNA thermometer, the fourU element. This element is found in several heat shock genes and other virulence genes (Hoe et al. 2013) important in intracellular pathogens.

## 8. RNome in Gram Positive Bacteria

*Enterococcus* spp.

In *Enterococcus* spp. the application of intergenic tiling microarrays allowed the identification of more than 75 new sRNA candidates under different stress conditions in *Enterococcus faecalis* (Shioya et al., 2011).

*Clostridium spp.*

Among *Clostridium* species, 159 sRNAs were described in *Clostridium difficile* and 46 sncRNAs were detected and confirmed using various methods (Soutourina et al. 2013). In addition, several sRNAs have been related to tolerance to elindamycin in *C. acetobutylicum* (Venkataramanan et al. 2013), while in *C. perfrigens* and *C. difficile* 94 trans-acting sRNAs and 92 cis-acting sRNAs have been described, including 16 di-cyclic-GMP responsive riboswitches ((Andre et al. 2008, Chen et al. 2011).

*Staphylococcus aureus:*

In *S. aureus*, several ncRNAs have been identified as also the peculiarity of "antisense regulatory mechanisms in *S. aureus*" (ARMSA). The transcriptome map of *S. aureus* has been completed. It contains 2912 transcripts, including 1554 mRNAs, 218 small non-coding RNAs and 1140 antisense RNAs. It also includes several overlapping transcripts (71 5' UTRs and 263 3' UTRs). In line with the findings, the transcriptome map also revealed a massive antisense transcription process that affect at least 50% of the transcripts. The transcriptome maps of isogenic mutants in ribonucleases (RNase III and PNP) and sigma B transcription factor have been compared with that of the wild-type strain. The results have uncovered the existence of a mechanism of digestion of overlapping transcripts by RNase III enzyme. As a consequence, the transcriptome contains a collection of short (20 nucleotides in average) RNA molecules accumulating in every region where overlapping transcription occurs. These results showed that PNPase is not required for this process.

The transcriptome map of *S. aureus* during phage replication and pathogenicity islands (PI) expression has been analysed using tiling arrays (Pichon and Felden 2005). Several genes whose expression is affected after SOS induction, by the presence or absence of a phage or a PI. Several copies of the small RNAs sprA and sprE-G have been localised in the pathogenicity islands of *S. aureus.*

Deletion mutant strains of four small RNA (RsaA, RsaE, RsaG and RsaH) in three different genetic backgrounds of *S.*

*aureus* (RN6390, HG001, Newman) have been successfully generated (Pichon and Felden 2005, Geissmann et al. 2009, Felden et al. 2011). The expression of some of Rsa RNAs varies in COL and RN6390. Five *rsa* genes (*rsaB, C, F, G* and *J*) were found specific to *S. aureus* whereas all the others (*rsaA, D, E, H, I* and *K*) are conserved in four different staphyloccocal species (*S. aureus, S. epidermidis, S. saprophyticus* and *S. haemolyticus*) (Geissmann et al. 2009). Analysis of the phenotypes associated to each small RNA reveal that RsaA is involved in biofilm and capsule formation whereas RsaG seems to be involved in fructose metabolism.

One of the largest bacterial regulatory ncRNAs is the *S. aureus* RNAIII. RNAIII is a 514 nt regulatory RNA that controls the switch between production of cell wall adhesins and synthesis of extracellular proteins, able to modulate the expression of genes involved in virulence at the post-transcriptional level (Guillet et al. 2013). A direct regulatory effect of RNAIII has been shown for *hla* and for *spa* expression (Mraheil et al. 2010). Romby's group (Romilly et al. 2012) focused on the interaction of RNAIII with several of its target mRNAs that encode virulence factors and one that encodes a transcription factor, Rot. The RNAIII–rot mRNA interaction was investigated, revealing the existence of several loop–loop interactions that partly overlap the ribosome binding site (rbs), thereby inhibiting ribosome binding. Furthermore, through a mechanisms resembling that of DsrA/rpoS regulation, the RNAIII–rot interaction creates an RNase III cleavage site within the rot mRNA, leading to its degradation. In addition, several novel ncRNAs were recently identified that might act as regulatory antisense RNAs (Lasa et al. 2011; Guillet et al. 2013). Sigma B-dependent RsaA RNA is a conserved small RNA in *S. aureus* functioning as a virulence suppressor of acute infections (Romilly et al. 2014). RsaA was shown to repress the synthesis of the global transcriptional regulator MgrA by forming an imperfect duplex with the Shine and Dalgarno sequence and a loop-loop interaction within the coding region of the target mRNA. Consequently, RsaA causes enhanced production of biofilm and a decreased synthesis of capsule formation in several strain backgrounds. RsaA attenuates the severity of acute systemic infections and favour chronic infections.

*Listeria monocytogenes*

Several approaches have been explored to search for ncRNAs in pathogenic *Listeria* spp. and reported on the determination of their regulatory networks. The Cossart 's team (2008, 2011) designed a method based on tiling arrays to look for ncRNAs in *L. monocytogenes*, an important foodborne pathogen. By comparing transcripts of strains grown under various conditions, 29 novel ncRNAs were identified, some of which follow the same expression pattern as virulence genes. The study revealed the existence of long antisense RNAs that span several genes located on the opposite strand, one of which negatively regulates genes required for flagellum synthesis genes in the flagellum locus. This antisense RNA is also used as an mRNA coding for the MogR repressor of flagella (Gripenland et al. 2010, Mandin and Guillier 2013).

RNA thermometers have been involved in regulation of virulence gene expression in *L. monocytogenes* (Loh et al. 2009). The transition from survival in the soil to virulence in human bodies is given by the sensing of temperature stress. PrfA is a general virulence gene activator, that possesses a 127 long secondary structure in the 5'-UTR. PrfA is folded into a stem-loop structure at lower temperatures, preventing its translation. At 37℃, the structure is opened and the PrfA product may orchestrate the synthesis of immune modulating factors, adhesins, and phagosome escape factors.

Furthermore, studying different strains led to the discovery of several single nucleotide polymorphisms and a phylogenesis of various disease causative agents. The first two genomes published belong to EGD and 10403S strains (Becavin et al. 2014). The differences between these reference strains consist in 317 SNPs, making these strains highly close. A new NGS work released the genome sequence of EGD-e, uncovering precious information on its peculiarities. EGD-e genome carries a point mutation in the PfrA gene, a transcriptional activator, leading to constitutive expression of several major virulence genes. Totally, EGD-e differs from EGD by 29016 SNPs, and from 10403S by 30296 SNPs, and is more related to serotype 1/2c than to 1/2a strains (Mraheil et al. 2010).

Serotypes 1/2a, 1/2b and 4b are most frequently associated to human infections. Hahn reported further genomic sequences, from strains CLIP80459, F2365 and L99, a virulence attenuated strain. The classification of these strains was made according to the serotype and to lineage. Serotype 1/2a (i.e. EGD) was related to lineage II, serotype 4b (CLIP80459) was related to lineage I, and serotype 4a related to lineage III (L99) (Hein et al. 2012).

150 putative sncRNAs, the major part acting in *trans*, were recently analysed in *L. monocytogenes* under extracellular and intracellular growth conditions, classified as 88 sRNAs, 29 antisense RNAs, and 33 cis-regulating elements (Gripenland et al. 2010, Mraheil et al., 2011).

Subversion of interferon (IFNs) response and *Interferon Stimulating Genes* (*ISGs*) by *Listeria* spp.

Pathogenic strains of *Listeria* are able to escape the immune response by deregulating the interferon signaling. The role of type I-IFNs in increasing host susceptibility could be explained by modulation of components of the immune response involved in controlling bacterial growth such as induction of T cell apoptosis, resulting in greater IL-10 secretion by phagocytic cells, in turn dampening the innate immune response (Dussurget et al. 2014). Different strains of *L. monocytogenes* have been shown to vary greatly in their capacity to induce IFN-ß (Reutterer et al., 2008; Schwartz et al., 2012). The LO28 strain hyper-induces IFN-ß (Reutterer et al., 2008). This strain bears a non-functional BrtA (also named TetR), the transcriptional repressor of the multidrug efflux pump MdrT (Schwartz et al., 2012;

Yamamoto et al., 2012). In *Listeria*, MdrT allows secretion of c-di-AMP, which triggers IFN-ß. Thus, derepression of MdrT in the LO28 strain promotes IFN-ß production. Of note, high expression of MdrT in LO28 correlates with both induction of IFN-ß and lower virulence. Another *Listeria* multidrug resistance transporter, MdrM, has been involved in the stimulation of IFN-ß production, possibly by secreting c-di-AMP (Crimmins et al., 2008; Woodward et al., 2010; Witte et al., 2012).

*Listeria* spp. evolved several mechanisms to avoid immune detection and evade IFN responses. It has been demonstrated that deacetylation of *Listeria* peptidoglycan by the deacetylase PgdA confers resistance to host lysozyme, thus preventing release of MAMPs, such as DNA, RNA and lipopeptides, that trigger IFN-ß production (Boneca et al., 2007). *Listeria* pgdA mutants are rapidly killed in murine macrophages, which produce lysozyme, and induce a strong secretion of IFN-ß compared to wild-type *Listeria*. The role of PgdA is not limited to the control of type I-IFN production: a pgdA mutant hyperinduces pro-inflammatory cytokines. Modification of peptidoglycan by PgdA is an extremely efficient mechanism of immune escape used by *Listeria*, which correlates with its critical role in virulence. *Listeria* has evolved a sophisticated strategy to modulate, either negatively or positively, the expression of ISGs in epithelial cells, by targeting BAHD1, a chromatin-repressive complex (Bierne et al., 2009; Lebreton et al., 2011, 2012). Indeed, *Listeria* infection promotes, albeit via an unknown mechanism, the targeting of BAHD1 at the promoter of a set of *ISGs*, thereby downregulating type I- and type III-IFN responses. *Listeria* can produce a nucleomodulin, LntA, which when secreted by intracellular bacteria, enters the nucleus of infected cells, binds BAHD1 and inhibits its function (Lebreton et al. 2012, Lebreton et al. 2014). Thus, LntA stimulates IFN responses. Consistent with the presence of HDAC1/2 in the BAHD1-associated complex, the level of acetylation of lysine 9 on histone H3, which is a mark of active chromatin, increases at the promoters of *ISGs* in the presence of LntA. When, in which host conditions, and how LntA targets BAHD1 specifically at *ISGs* remains an open question. The LntA-mediated stimulation of type III-IFN responses might support localized pro-bacterial conditions, as was proposed for IFN-I responses (Toledo-Arana et al. 2009; Dussurget et al. 2014). In addition to suggesting a role for non-coding RNA in virulence of *L. monocytogenes*, new findings (Wurtzel et al. 2012) identified a recurrent organization, called excludon, where transcription of long RNAs, antisense to transcripts encoding proteins, represses expression of overlapping genes whose functions are opposite.

Subversion of immune response-related microRNAs by *Listeria* spp.

A recent study showed altered immune response of mice deficient in miR-155 to the facultative intracellular pathogen *Salmonella* (Schulte et al. 2011). IL-6 and IL-10 were found regulated by miRNAs of the let-7 family and miR-155 induction by secreted effector proteins of *Salmonella*. Caco-2 cells were infected with *Listeria monocytogenes* EGD-e, a mutant strain (*ΔinlAB* or *Δhly*) or incubated with purified listeriolysin (LLO). Infection with wild-type bacteria led to significantly increased expression of miR-146b, miR-16 and miR-155 in Caco-2 cells compared to non-infected cells (Izar et al. 2012). Significant changes of target mRNA levels were also observed in infected Caco-2 cells. miRNA expression profiling in human macrophages showed that miR-146 and miR-155 are endotoxin-responsive genes involved in several immune and inflammatory pathways. miR-146b was shown to inhibit IL-8 expression, possibly through interleukin-1 receptor-associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6), two major adaptor molecules in TLR receptor signaling and NF-kB activation. Thus, Izar and colleagues showed a mechanism exploited by *Listeria* through TLR receptors leading to upregulation of inflammatory microRNAs.

# 9. RNome in Gram Negative Bacteria

*Pseudomonas aeruginosa* sRNAs

The ferric uptake repressor Fur is a negative regulator of genes involved in iron acquisition or of iron-regulated genes involved in virulence (Vasil 2007). Fur exerts its positive effect by repressing a negative regulatory sRNA factor, PrrF. Other sRNAs belonging to the Csr/Rsm system are present and act with mechanisms similar to those of E- coli Csr system (Toledo-Arana et al. 2007).

*Salmonella* spp. and *E. coli*

A model organism for Gram-negative pathogens is *Salmonella typhimurium*. By using a co-immunoprecipitation approach in combination with 454 deep sequencing, Vogel (Vogel 2009, Storz et al. 2011) detected more than 100 Hfq-binding ncRNAs that are encoded in intergenic regions. To identify potential target mRNAs controlled directly by ncRNAs, pulse-expression studies followed by transcriptome analyses were then performed. These studies indicated that several hundreds (or >20%) of all *Salmonella* genes are regulated by Hfq and ncRNAs (Sittka et al, 2008).

OmrA and OmrB are two Hfq-dependent sRNAs encoded by adjacent genes; their 5' and 3' ends are the most well conserved regions among enterobacteria, and are almost identical between OmrA and OmrB. Several genes, that are targeted by the conserved 5' end of OmrA or OmrB, have been identified, and their function is often related to the cell surface. They encode outer membrane proteins (e.g. OmpT, CirA, FecA and FepA), as well as CsgD and FlhDC, two transcriptional master regulators of motility and biofilm formation that respond to multiple environmental cues. csgD regulates curli synthesis, while flhDC regulates genes involved in flagellar synthesis and assembly. csgD and flhDC expression is controlled at the transcription level by a variety of transcriptional regulators, and at the

posttranscriptional level via multiple Hfq-dependent sRNAs (Mandin and Guillier 2013).

The tight control by sRNAs of the amount of proteins or appendages that are recognized by eukaryotic cells during infection could sustain bacterial virulence. OmrA and OmrB also directly repress the synthesis of their transcriptional regulator, the EnvZ-OmpR two-component system, which creates a negative feedback loop through which OmrA and OmrB can repress their own expression. GcvB, a pleiotropic sRNA, was recently identified as a sRNA regulator of the central PhoQ-PhoP Two-Component -System (TCS) in *E. coli* (Coornaert et al. 2012). CRP (cAMP receptor protein) is the global regulator of catabolite repression and it regulates >100 genes, including several sRNAs, such as: McaS and CyaR, two regulators of group behavior.

During the course of infection, enteric bacteria have to respond and adapt to changing environmental conditions at the sites of invasion. To this aim, bacteria have developed complex regulatory processes for the temporal and spatial control of virulence factor expression, exploiting sRNAs in the control of motility and biofilm formation (Mika and Hengge 2013).

In enterohemorrhagic *E. coli* (EHEC), two small RNAs, GlmY and GlmZ, have been shown to exert a coordinated action that leads to pathogenesis. These sRNAs act as dimers to direct the cleave of the transcript containing *espJ* and *EspFu*, thus enabling the translation of EspFu protein that allows mammalian cell invasion by the production of pedestal structures and docking on the enterocytes. *E. coli* exploits type III secretion system (T3SS) to inject proteins into epithelial cells and require the pathogenicity island named *locus of enterocyte effacement* (*LEE*) that encodes for the molecular syringe components. *LEE* operon was shown to be dependent on a functional CsrA activity (Bhatt et al. 2009). CsrA mutants showed reduced pedestal formation, ineffective translocation of EspA, EspB, EspD, EscF and the effector Translocated intimin receptor (Tir), that accumulates inside the bacteria and cannot be transferred by the T3SS system. GlmY and GlmZ, in one case, promote EspFu expression and translation by cleavage of the transcript, while, in the case of LEE, promote destabilization of the mRNA (Gruber and Sperandio 2014). GlmY and GlmZ act in concert to destabilise the LEE4 and LEE5 transcripts (Papenfort and Vogel 2014). In another context, GlmY and GlmZ were found involved in metabolism control, regulating the synthesis of amino sugars such as glucosamine, exposing the ribosome binding site of the *glmS* gene (Urban and Vogel 2008). GlmZ binds directly to *glmS* with the guide of Hfq, while GlmY is an anti-adaptor RNA that protect GlmZ from RapZ, an adaptor protein that guides RNase E to GlmZ for cleavage (Gopel et al. 2013); GlmY is a decoy that through molecular mimicry and a secondary structure similar to GlmZ attracts RapZ and make it unavailable for its function as RNase adaptor protein for small RNA GlmZ.

# 10. Conclusions

In this review several novel cases of small RNAs regulating phase transition and response to the environment (acid stress, pH, temperature switch, oxygen, osmotic stress) were discussed. It is expected that the knowledge on bacterial small RNAs will continue to evolve. It is highly expected that scientists will provide new information and perform advancements in science of pathogen containment through exploitation of RNA aptamers, plasmids and bacteriophages counterfighting the activity of the small RNAs and RNA sequences in the genomes of pathogens.

# REFERENCES

[1] Andre G., Even S., Putzer H., Burguiere P., Croux C., Danchin A., Martin-Verstraete I., Soutourina O. S-box and T-box riboswitches and antisense RNA control a sulfur metabolic operon of *Clostridium acetobutylicum*. Nucl. Acids Res., 2008, 36:5955–5969.

[2] Babitzke P. and Romeo T. CsrB sRNA family: sequestration of RNA-binding regulatory proteins. Curr. Opin. Microbiol., 2007, 10(2):156-163.

[3] Becavin C., Bouchier C., Lechat P., Archambaud C., Creno S., Gouin E., Kuhbacher A., Pucciarelli M.G., Garcia-del Portillo F., Hain T., Portnoy D.A., Chakraborty T., Lecuit M., Pizarro-Cerdà J., Moszer I., Bierne H. Cossart P. Comparison of widely used Listeria monocytogenes strains EGD, 10403S, and EGD-e highlights genomic variations underlying differences in pathogenicity. Mbio, 2014, 5:e00969-14.

[4] Bertrand R., Schuster C.F. Post-transcritional regulation of gene expression in bacterial pathogens by toxin-antitoxin systems. Front. Cell. Infect. Microbiol., 2014, 4:6.

[5] Bordeau V. and Felden B. Curli synthesis and biofilm formation in enteric bacteria are controlled by a dynamic small RNA module made up of a pseudoknot assisted by an RNA chaperone. Nucl. Acids Res., 2014, 42:4682-4692.

[6] Chen X., Taylor D.W., Fowler C.C., Galan J.E., Wang H.W., Wolin S.L. An RNA degradation machine sculpted by Ro autoantigen and noncoding RNA. Cell, 2013, 153:166–177.

[7] Coornaert A., Chiaruttini C., Springer M. and Guillier M., Post-transcriptional control of the *Escherichia coli* PhoQ-PhoP two-component system by multiple sRNAs involves a novel pairing region of GcvB. PLoS Genetics, 2012, 9:e1003156.

[8] De Lay N., Schu D.J., Gottesman S. Bacterial small RNA-based negative regulation: Hfq and its accomplices. J. Biol. Chem., 2013, 288:7996-8003.

[9] Durand S, Jahn N, Condon C, Brantl S. Type I toxin-antitoxin systems in *Bacillus subtilis*. RNA Biol. 2012, 9(12):1491-7.

[10] Duss O., Michel E., Yulikov M., Schbert M., Jaeschke G., Allain F.H. Structural basis of the non-coding RNA RsmZ

acting as a protein sponge. Nature, 2014a, 509:588-592.

[11] Duss O., Michel E., Diarra Dit Konté N., Schbert M., Allain F.H. Molecular basis for the wide range of affinity found in Csr/Rsm protein-RNA recognition. Nuc. Acids Res., 2014b, 42:5332-5346.

[12] Dussurget O., Bierne H. and Cossart P. The bacterial pathogen *Listeria monocytogenes* and the interferon family: type I, type II and type III interferons. Front. Cell. Infect. Microbiol., 2014, 4:50.

[13] Engelberg-Kulka H., Hazan R., Amitai S. mazEF: a chromosomal toxin-antitoxin module that triggers programmed cell death in bacteria. J Cell Sci., 2005, 118:4327-4332.

[14] Felden B., Vandenesch F., Bouloc C., Romby P. The Staphylococcus Aureus RNome and its commitment to virulence. PLoS Pathogen, 2011, 7:e1002006.

[15] Goeders N., Van Melderen L. Toxin-antitoxin systems as multilevel interaction systems. Toxins (Basel), 2014, 10:304-324.

[16] Fozo E.M. New type I toxin-antitoxin families from "wild" and laboratory strains of *E. coli*: Ibs-Sib, ShoB-OhsC and Zor-Orz. RNA Biol., 2012, 9:1504-1512.

[17] Fozo EM, Hemm MR, Storz G. Small toxic proteins and the antisense RNAs that repress them. Microbiol. Mol. Biol. Rev., 2008, 72(4):579-89.

[18] Fozo EM, Makarova KS, Shabalina SA, Yutin N, Koonin EV, Storz G. Abundance of type I toxin-antitoxin systems in bacteria: searches for new candidates and discovery of novel families. Nucl. Acids Res., 2010, 38(11):3743-59.

[19] Geissmann T., Chevalier C., Cros M.-J., Boisset S., Fechter P., Noirot C., Schrenzel J., Francois P., Vandenesch F., Gaspin C., Romby P. A search for small noncoding RNAs in *Staphylococcus aureus* reveals a conserved sequence motif for regulation. Nucl. Acids Res., 2009, 37:7239–7257.

[20] Gopel Y., Papenfort K., Reichenbach B., Vogel J., Gorke B. Targeted decay of a regulatory small RNA by an adaptor protein for RNase III and counteraction by an anti-adaptor RNA. Genes Develop., 2013, 27:552-564.

[21] Gripenland J., Netterling S., Loh E., Tiensuu T., Toledo-Arana A., Johansson J. RNAs: regulators of bacterial virulence. Nat. Rev. Microbiol. , 2010, 8:857-866.

[22] Gruber C.C. and Sperandio V. Posttranscriptional control of microbe-induced rearrangement of host cell actin. MBio, 2014, 5(1):e01025-13.

[23] Guillet J.., Hallier M.., Felden B. Emerging functions for the *Staphylococcus aureus* RNome. PLoS Pathogens, 2013, 9:e1003767.

[24] Hain T., Ghai R., Billion A., Kuenne C.T., Steinweg C., Izar B., Mohamed W., Mraheil M.A., Domann E., Schaffrath S., Kärst U., Goesmann A., Oehm S., Pühler A., Merkl R., Vorwerk S., Glaser P., Garrido P., Rusniok C., Buchrieser C., Goebel W., Chakraborty T. Comparative genomics and transcriptomics of lineages I, II, and III strains of *Listeria monocytogenes*. BMC Genomics, 2012, 13:144.

[25] Hoe C.-H., Raabe C.A., Rozhdestvensky T.S., Tang T.-H. Bacterial RNAs: regulation in stress. Int. J. Med. Microbiol., 2013, 303:217-229.

[26] Izar B., Mraheil M.A., Hain T. Identification and role of regulatory non-coding RNAs in *Listeria monocytogenes*. Int. J. Mol. Sci., 2011, 12:5070-5079.

[27] Izar B., Mannala G.K., Mraheil M.A., Chakraborty T., Hain T. microRNA Response to Listeria monocytogenes Infection in Epithelial Cells. Int. J. Mol. Sci., 2012, 13(1):1173-1185.

[28] Jahn N, Brantl S. One antitoxin--two functions: SR4 controls toxin mRNA decay and translation. Nucleic Acids Res., 2013, 41(21):9870-9880.

[29] Kawano M. Divergently overlapping cis-encoded antisense RNA regulating toxin-antitoxin systems from E. coli: hok/sok, ldr/rdl, symE/symR. RNA Biol., 2012, 9(12): 1520-1527.

[30] Kawano M, Aravind L, Storz G. An antisense RNA controls synthesis of an SOS-induced toxin evolved from an antitoxin. Mol Microbiol., 2007, 64(3):738-754.

[31] Krupovic M., Makarova K.S., Forterre P., Prangishvili D. and Koonin E.V. Casposons: a new superfamily of self-synthesizing DNA transposons at the origin of prokaryotic CRISPR-Cas immunity. BMC Biology, 2014, 12:36.

[32] Lasa I., Toledo-Arana A., Dobin A., Villanueva M., Ruiz de los Mozos I., Vergara-Irigaray M., Segura V., Fagegaltier D., Penades J.R., Valle J., Solano C., Gingeras T.R. Genome-wide antisense transcription drives mRNA processing in bacteria. Proc. Natl. Acad. Sci. USA, 2011, 108:20172-20177.

[33] Loh E., Dussurget ,O., Gripenland ,J., Vaitkevicius K., Tiensuu T., Mandin P., Johansson J. A trans-acting riboswitch controls expression of the virulence regulator PrfA in *Listeria monocytogenes.* Cell, 2009, 139:770–779.

[34] Makarova KS, Wolf YI, Koonin EV: The basic building blocks and evolution of CRISPR-Cas systems. Biochem. Soc. Trans., 2013, 41:1392-1400.

[35] Mandin P., Repoila F., Vergassola M., Geissmann T., Cossart P. Identification of new noncoding RNAs *in Listeria monocytogenes* and prediction of RNA targets. Nucl. Acids Res., 2007, 35:962-975.

[36] Mandin P. and Guillier M. Expanding control in bacteria: interplay between small RNAs and transcriptional regulators to control gene expression. Curr. Opin. Microbiol., 2013, 16, 125–132.

[37] Mraheil M.A., Billion A., Kuenne C., Pischimarov J., Kreikemeyer B., Engelmann S., Hartke A., Giard J.-C., Rupnik M., Vorwerk S., Beier M., Retey J., Hartsch T., Jacob A., Cemic F., Hemberger J., Chakraborty T., Hain T. Comparative genome-wide analysis of small RNAs of major Gram-positive pathogens: from identification to application. Microbial Biotechnol., 2010, 3:658-676.

[38] Mraheil M.A., Billion A., Mohamed W., Mukherjee K., Kuenne C., Pischimarov J., Krawitz C., Retey J., Hartsch T., Chakraborty T., Hain T. The intracellular sRNA trancriptome of Listeria monocytogenes during growth in macrophages. Nucl. Acids Res., 2011, 39:4235-4248.

[39] Mika F. and Hengge R. Small Regulatory RNAs in the control of motility and biofilm formation in *E. coli* and

*Salmonella*. Int. J. Mol. Sci., 2013, 14:4560-4579.

[40] Nechooshtan G., Elgrably-Weiss M., Sheaffer A., Altuvia S. et al. A pH-responsive riboregulator. Genes Dev., 2009, 23: 2650-2662.

[41] Papenfort K., Vogel J. Small RNA functions in carbon metabolism and virulence of enteric pathogens. Front. Cell. Infect. Microbiol., 2014, 4:91.

[42] Pichon C. and Felden B. Small RNA genes expressed from *Staphylococcus aureus* genomic and pathogenicity islands with specific expression among pathogenic strains. Proc. Natl. Acad. Sci. USA, 2005, 102:14249–14254.

[43] Resch A., Afonyushkin T., Lombo T.B., McDowall K.J., Bläsi U., Kaberdin V.R. Translational activation by the noncoding RNA DsrA involves alternative RNase III processing in the rpoS 5'-leader. RNA, 2008, 14(3):454-459.

[44] Romeo T., Vakulskas C.A., Babitzke P. Post- transcriptional regulation on a global scale: form and function of Csr/Rsm systems. Environ. Microbiol. 2013, 15:313-324.

[45] Romilly C., Calderari I., Parmentier D., Lioliou E., Romby P., Fechter P. Current knowledge on regulatory RNAs and their machineries in *Staphylococcus aureus*. RNA Biology, 2012, 9:402-413.

[46] Romilly C., Lays C., Tomasini A., Caldelari I., Benito Y., Hammann P., Geissmann T., Boisset S., Romby P., Vandenesch F. A non-coding RNA promotes bacterial persistence and decreases virulence by regulating a regulator in *Staphylococcus aureus.* PLoS Pathog., 2014, 10:e1003979.

[47] Schulte L.N.; Eulalio A.; Mollenkopf H.J.; Reinhardt R.; Vogel J. Analysis of the host microRNA response to Salmonella uncovers the control of major cytokines by the let-7 family. EMBO J., 2011, 30:1977–1989.

[48] Sesto N., Touchon M., Andrade J.M., Kondo J., Rocha E.P., Arraiano C.M., Archambaud C., Westhof E., Romby P., Cosssart P. A PNPase dependent CRISPR system in *Listeria*. PloS Genet., 2014, 10:e1004065.

[49] Shioya K., Michaux C., Kuenne C., Hain T., Verneuil N., Budin-Verneuil A., Hartsch T., Hartke A., Giard J.C. Genome-wide identification of small RNAs in the opportunistic pathogen *Enterococcus faecalis* V583. PLoS One, 2011, 6(9):e23948.

[50] Sittka A., Lucchini S., Papenfort K., Sharma C.M., Rolle K., Binnewies T.T., Hinton J.C., Vogel J. Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. PLoS Genet., 2008, 4(8):e1000163.

[51] Soutourina O.A., Monot M., Boudry P., Saujet L., Puchon C., Sismeiro O., Semenova E., Severinov K., Le Bouguenec C., Coppée J.Y., Dupuy B., Martin-Verstraete I. Genome-wide identification of regulatory RNAs in the human pathogen *Clostridium difficile*. PLoS Genet., 2013, 9:e1003493.

[52] Storz G., Vogel J., Wasssarman K.M. Regulation by small RNAs in bacteria: expanding frontiers. Mol. Cell, 2011, 43:880-891.

[53] Syed M.A.and Lévesque C.M. Chromosomal bacterial type II toxin-antitoxin systems. Can. J. Microbiol., 2012, 58:553-562.

[54] Toledo-Arana A., Repoila F. and Cossart P. Small noncoding RNAs controlling pathogenesis. Curr. Op. Microbiol., 2007, 10:182-188.

[55] Toledo-Arana A., Dussurget O., Nikitas G., Sesto N., Guet-Revillet H., Balestrino D., Loh E., Gripenland J., Tiensuu T., Vaitkevicius K. et al.: The *Listeria* transcriptional landscape from saprophytism to virulence. Nature, 2009, 459:950-956.

[56] Unterholzner S.J., Poppenberger B., Rozhon W. Toxin-antitoxin systems: Biology, identification, and application. Mob. Genet. Elements, 2013, 3(5):e26219.

[57] Urban J. and Vogel J. Two seemingly homologous noncoding RNAs act hierarchically to activate glmS mRNA translation. PLoS Biol., 2008, 6(3):e64.

[58] Venkataramanan K.P., Jones S.W., McKormick K.P., Kunjeti S.G., Ralston M.T., Meyers B.C., Papoutsakis E.T. The *Clostridium* small RNome that responds to stress: the paradigm and importance of toxic metabolite stress in *C. acetobutylicum*. BMC Genomics, 2013, 14:849.

[59] Vogel J. A rough guide to the non-coding RNA world of *Salmonella*. Mol. Microbiol. 2009, 71:1-11.

[60] Wang X., Wood T.K. Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. Appl. Environ. Microbiol., 2011, 77:5577-5583.

[61] Wang X., Lord S.H., Hong S.H., Peti W., Benedik MM.J., Page R., Wood T.K. Type II toxin/antitoxin MqrS/MqsA controls type V toxin/antitoxin GhoT/GhoS. Environm. Microbiol., 2013, 15:1734-1744.

[62] Wen J., Fozo E.M. sRNA Antitoxins: more than one way to repress a toxin. Toxins (Basel), 2014, 6(8):2310-35.

[63] Wen J., Won D., Fozo E.M. The ZorO-OrzO type I toxin-antitoxin locus: repression by the OrzO antitoxin. Nucleic Acids Res., 2014, 42(3):1930-46.

[64] Westra E.R., Swarts D.C., Staals R.H.J., Jore M.M., Brouns S.J.J., van der Oost J: The CRISPRs, they are a-changin': how prokaryotes generate adaptive immunity. Annu. Rev. Genet., 2012, 46:311-339.

[65] Wurtzel O., Sesto N., Mellin J.R., Karunker I., Edelheit S., Becavin C., Archambaud C., Cossart P., Sorek R: Comparative transcriptomics of pathogenic and non-pathogenic *Listeria* species. Mol. Syst. Biol., 2012, 8:583.

[66] Zorzini V., Buts L., Sleutel M., Garcia-Pino A., Talavera A., Haesaerts S., De Greve H., Cheung A., van Nuland N.A., Loris R. Structural and biophysical characterization of *Staphylococcus aureus* SaMazF shows conservation of functional dynamics. Nucleic Acids Res., 2014; 42(10):6709-25.